NEW RECORDS OF *BELLOTTIA APODA* (BYTHITIDAE) IN THE ADRIATIC SEA WITH NOTES ON MORPHOLOGY AND BIOLOGY

by

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ABSTRACT. - One hundred and twenty-eight specimens of *Bellottia apoda* Giglioli, 1883 were caught by bottom trawl in the Central Adriatic Sea between 1970 and 1994, at about 200 m depth. A subsample of 22 males and 20 females was selected for meristic counts and morphometric measurements. All the examined measurements show isometric growth, except for "head length" which exhibits negative allometry in both sexes. Sexual dimorphism in the relative growth of "preanal length" and "head length" is shown. Characteristics of gill arches and dentition highlighted both by SEM examination and analysis of stomach contents confirm that *B. apoda* is a carnivorous predator, feeding on both planktonic and benthic organisms. Observations on ovary maturity stages of 29 females caught all year round, and presence of hatching embryos in August sample, indicate late summer as the main reproductive period. The finding of spermatophores in ovaries in different developmental stages implies that females can store spermatophores until complete egg ripeness.

RÉSUMÉ. - Nouveaux signalements de *Bellottia apoda* (Bythitidae) dans la Mer Adriatique. Notes sur sa morphologie et sur sa biologie.

Un lot de 128 individus de *Bellottia apoda* Giglioli, 1883 a été capturé par chalutage, en Adriatique Centrale, pendant la période 1970-1994, à une profondeur de 200 m environ. Un échantillon de 22 mâles et de 20 femelles a été utilisé pour les données morphométriques et méristiques. La totalité des mesures morphométriques montre une croissance isométrique, à l'exception du paramètre "longueur de la tête" qui présente une allométrie négative dans les deux sexes. Le dimorphisme sexuel est mis en évidence dans la croissance relative de la "longueur préanale" et de la "longueur de la tête". Les caractéristiques des arcs branchiaux et de la denture, mises en évidence par l'observation au MEB, et l'analyse des contenus stomacaux confirment que *B. apoda* est un prédateur carnivore se nourrissant d'organismes benthiques et planctoniques. Les observations sur le stade de maturité des ovaires de 29 femelles capturées pendant toute l'année et la présence, dans l'échantillon d'août, d'embryons prêts à naître, indiquent que la fin de l'été est la principale période pour la ponte. L'hypothèse que les spermatophores peuvent être stockés par la femelle jusqu'à la maturation des oeufs est confirmée par leur découverte dans des ovaires à différents stades de maturation.

Key-words. - Bythitidae, *Bellottia apoda*, MED, Adriatic Sea, Morphology, SEM, Relative growth, Feeding, Reproduction.

Bellottia apoda Giglioli, 1883 is a small fish belonging to the family Bythitidae that contains all the viviparous genera formerly referred to the family Brotulidae (Cohen and Nielsen, 1978). Giglioli (1883) based his description of *B. apoda* on 2 specimens trawled in the Gulf of Naples, at depth of 30 m on *Posidonia*. These specimens were de-

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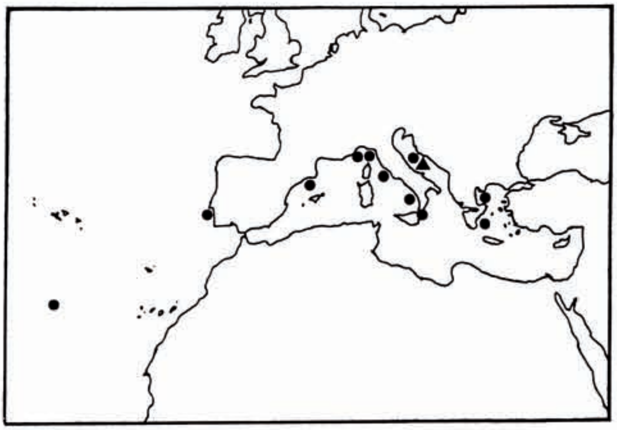

Fig. 1. - Geographical distribution of *Bellottia apoda* (black circles for literature records; black triangle for present material).

posited in the Zoological Museum of Florence University. In the same haul, 3 more specimens were collected, but not examined by Giglioli. Placed in other Italian Institutions, they were unfortunately lost during world war II. Nielsen and Cohen (1968) accurately redescribed the species from the 2 syntypes of Giglioli, still preserved in Florence.

There is no other record of *B. apoda* till 1976, when it was recorded by Maul in the East Atlantic (off Madeira and south Portugal) and by Relini Orsi in the Ligurian Sea. Subsequently the species was recorded in several Mediterranean localities: Greece, in the Saronikos Gulf (Papaconstantinou *et al.*, 1977); Sicily, a stranded specimen found near Messina (Berdar *et al.*, 1977); Central Adriatic (Jardas, 1979; Zupanovic and Jardas, 1989); southern France, near Villefranche-sur-Mer (Sardou, 1980); Spain, near Barcelona (Alluè, 1984); Greece, in the Euboikos Gulf (Papaconstantinou, 1984) and Italy, in the Tyrrhenian Sea, between Giglio and Montecristo Islands (De Ranieri and Sbrana, 1992).

The geographical distribution of the species, as presently known, is given in figure 1.

Until now, *B. apoda* has been considered a quite rare species, always caught in one or few specimens. The largest sample was that of Papaconstantinou *et al.* (1977), with 8 specimens.

In May 1970, two specimens of *B. apoda* were collected in the Western Pomo pit (Central Adriatic) by Dr. C. Froglia, who continued to gather other material in subsequent years. Berdar (1977) mentioned 8 specimens collected until 1977 in the Adriatic. From then on, a total of 128 specimens was collected in the area. At present 121 specimens are preserved in the Ichthyological Reference Collection of I.R.PE.M. (Istituto Ricerche Pesca Marittima) in Ancona. Two (1 male and 1 female) were given to the US National Museum, Smithsonian Institution, Washington DC, and five, badly damaged, have been discarded. This rather abundant material allowed us to study some details of less investigated biological and morphological characteristics of the species.

MATERIAL AND METHODS

All the specimens were fished in the Western part of Pomo pit by bottom trawl, on epibathyal muds at depths ranging from 180 to 256 m. This area has frequently been sampled because it is the main "deep water" *Nephrops* ground in the Central Adriatic (Froglia and Gramitto 1981, 1988). Even though fishing operations were carried out in all the periods of a year and in diel cycles, *Bellottia apoda* was always collected in night tows.

The specimens were fixed in 5% formol saline solution and then stored in 70% alcohol for preservation. All were sexed on the basis of secondary sexual characters and a subsample of 42 undamaged fishes (22 males and 20 females) was chosen for morphological analysis.

Meristic characters and morphometrical measurements were recorded using a stereomicroscope with an ocular micrometer. In morphometrical analysis 4 males were discarded, being bent or fixed with open mouth.

To compare relative growth of males and females, the linear regression ($Y = \underline{a} + \underline{b}X$) was calculated on log transformed data. Covariance analysis was then performed on regression lines, testing homogeneity of slopes and of elevations by F-test (Snedecor and Cochran, 1967). Departure from isometry ($\underline{b} = 1$) was tested by t-test.

In order to clarify some interesting morphological characteristics, dentary bones and gill arches were examined with SEM. They were dehydrated in an alcohol series, critical-point dried in a BioRad E 3100 dryer, sputter-coated with gold (2 min at 20 mA) and examined with a JEOL JSM 5200 scanning electron microscope.

A sample of fishes caught in different seasons was dissected to collect data on feeding and reproduction of the species.

For stomach contents analysis three indices were used on 24 specimens: Vacuity index (V%); Frequency of occurrence (F%); and Mean number of item x ($\overline{N}_x$). These numerical indices, commonly used in stomach content analysis (Sorbe, 1980) identify the basic component of the diet of a species in an immediate and enough accurate way (Hyslop, 1980).

On the basis of oocyte diameter and general aspect of ovary observed in 29 females, 4 stages of maturity were defined, corresponding to 4 periods of the reproductive cycle of *B. apoda*:

- stage 1, quiescent period: ovaries have not yet started developing; oocytes numerous and very small (less than 500 µm in diameter);
- stage 2, vitellogenic period: developing ovaries with eggs visible to naked eye (diameter 500-800 µm), or ovaries in advanced ripeness with very large eggs (diameter 800-1200 µm);
- stage 3, gestation period: ovaries with embryos in a more or less advanced stage of development;
- stage 4, recovery period: after birth; ovaries as empty sacs, usually containing residuals of spermatophores and few, undeveloped oocytes.

Presence of spermatophores was also noted.

RESULTS

General morphology of *B. apoda* has been already described by many authors (i.e., Nielsen and Cohen, 1968; Papaconstantinou *et al.*, 1977; Sardou, 1980; Alluè, 1984). Therefore we give the basic meristic (Table I) and morphometric (Table II) characteristics of our specimens.

A 25.9 mm male is the smallest among our specimens and also the smallest male ever recorded. Our largest specimen is a female (48.5 mm) but larger specimens have been reported earlier (Maul, 1976; Papaconstantinou *et al.*, 1977; Jardas, 1979; Papaconstantinou, 1984).

Nineteen specimens out of 42 examined had parasitic Copepoda of family Caligidae on fins.

Morphometrics

Comparing the regression equations of males and females (Table III), significant differences in growth were found in the "head" and "preanal" length. Therefore it is not justified to combine the two sexes in analyses aimed at testing the existence of allometric growth (Table III) in these two characters.

In details, both "predorsal length" and "max. body depth" exhibit a sex-undifferentiated, isometric growth model. "Preanal length" and "head length" show a different growth between males and females, but the last is the only one exhibiting negative allometry in both sexes.

Table I. - Meristic characters of *Bellottia apoda.*

	Males (n = 22)		**Females (n = 20)**	
	min - max	**mean ± s.d.**	**min - max**	**mean ± s.d.**
Standard length (mm)	25.9 - 42.8	35.7 ± 4.0	32.0 - 48.5	36.6 ± 4.1
Dorsal rays	81 - 95	86.7 ± 3.4	81 - 96	88.1 ± 3.9
Anal rays	65 - 76	71.4 ± 3.2	64 - 76	72.5 ± 3.1
Caudal rays	6 - 6	6.0 ± 0.0	6 - 6	6.0 ± 0.0
Pectoral rays (right)	21 - 23	22.3 ± 0.6	21 - 24	22.0 ± 1.0
Pectoral rays (left)	22 - 23	22.4 ± 0.5	21 - 24	22.0 ± 0.9

Table II. - Morphometric characters (in % of SL) of *Bellottia apoda.*

	Males (n = 18)		**Females (n = 20)**	
	min - max	**mean ± s.d.**	**min - max**	**mean ± s.d.**
Standard length (mm)	25.9 - 42.8	35.4 ± 4.3	32.0 - 48.5	36.6 ± 4.1
Predorsal length	33.6 - 41.3	38.5 ± 1.8	34.4 - 40.9	37.6 ± 1.9
Preanal length	48.1 - 55.3	51.5 ± 2.0	46.4 - 52.5	50.1 ± 1.7
Head length	26.8 - 30.8	28.6 ± 1.2	24.7 - 29.0	27.0 ± 1.2
Upper jaw length	11.3 - 15.4	13.5 ± 1.3	11.2 - 16.2	13.2 ± 1.0
Horizontal diameter of orbit	4.0 - 5.3	4.7 ± 0.5	3.7 - 5.5	4.7 ± 0.5
Interorbital width	6.0 - 7.4	6.6 ± 0.4	5.8 - 7.8	6.5 ± 0.5
Maximum body depth	18.7 - 24.4	21.7 ± 1.3	19.4 - 23.1	21.5 ± 1.1

Gill arches

The examination under scanning microscope confirms the structure described by Nielsen and Cohen (1968) and Sardou (1980).

On each gill arch, the gill laminae with the two series of lamellae are clearly evident (Fig. 2a). Only the anterior gill arch is provided with two different types of gill rakers: 4 elongated, stick-shaped and 9 rounded, tubercle-shaped. The other arches show only tubercle-shaped gill rakers. Both types of rakers are covered with small, sharp teeth, more or less protuberant, which on the stick-shaped rakers are placed at the distal end of the "stick", on the inner side (Fig. 2b).

Dentition

Mouth is large, with strong jaws. Premaxillary bears large and slightly curved teeth on outer row and smaller straight teeth on two-three inner rows (Fig. 2c), which gradually decrease to a single row posteriorly. The dentaries bear pointed teeth arranged approximately in two rows, large on the inner and small on the outer one.

Palatine and vomerine teeth are small and pointed, arranged in ill-defined rows, arch-shaped on the widened anterior part of the vomer. In the roof of the mouth, scanning microscopy showed characteristic structures (Fig. 2d), similar to the taste buds described in many fish species (Hara, 1971; Gerking, 1994).

Numerous and small teeth are present on pharyngeal bones.

Feeding

The 24 specimens examined for stomach contents analysis had sizes ranging between 30 and 43 mm SL.

Results are given for all specimens pooled, with no reference to size and seasons (Table IV). Because of the poor condition of stomach contents, food items were grouped in large inclusive categories, although in some cases prey identification was possible at genus level.

Only 3 specimens had an empty stomach. As all our specimens were caught in night tows, the low value of vacuity index (12%) indicates that the species feeds by night.

In specimens with full stomachs the number of prey items varies between 1 and 13. Copepoda (Calanoida) and Amphipoda (generally Gammaridea) form the bulk of food

Table III. - *Bellottia apoda*. Summary of regression equations for each morphometrical character, F values for slopes (b) and elevations (a) comparison between males and females (* indicates F values statistically significant at $\alpha = 0.05$) and type of allometry (testing $b = 1$, at $\alpha = 0.05$).

	Sex	N	a	b	r	b comp. $F_{(1,34)}$	a comp. $F_{(1,35)}$	Allometry
Predorsal length	M	18	- 0.549	1.087	0.95			isometry
	F	20	- 0.389	0.977	0.90	0.577	2.318	isometry
Preanal length	M	18	- 0.301	1.008	0.96			isometry
	F	20	- 0.239	0.960	0.95	0.202	5.295 *	isometry
Head length	M	18	- 0.307	0.846	0.95			negative
	F	20	- 0.070	0.680	0.93	3.198	18.684 *	negative
Maximum body depth	M	18	- 0.790	1.081	0.92			isometry
	F	20	- 0.827	1.102	0.92	0.019	0.216	isometry

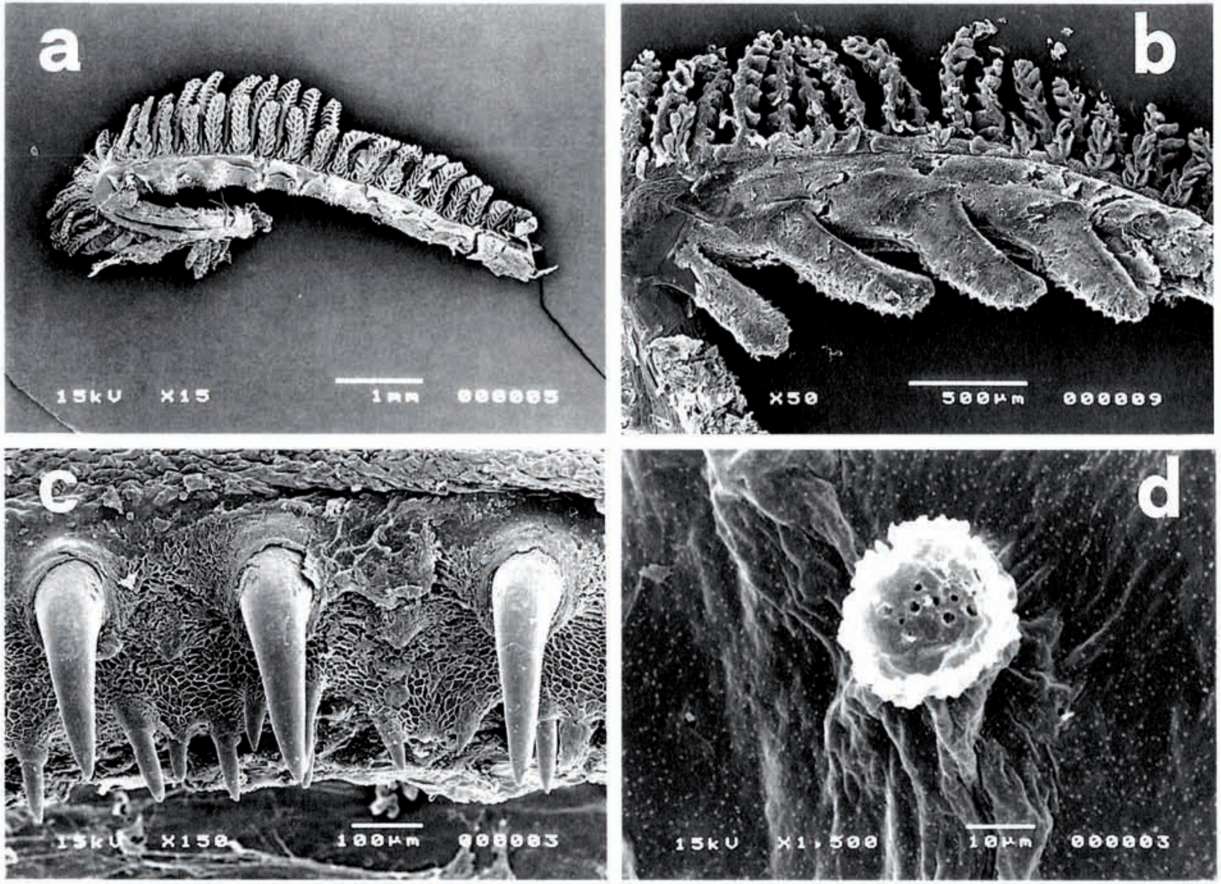

Fig. 2. - a) Second gill arch of *Bellottia apoda*. b) Detail of the first gill arch with the elongated stick-shaped rakers. c) Detail of premaxillary dentition. d) Detail of the mouth roof with one of the supposed taste buds.

(Table IV). Copepoda is by far the preferential food all year round, both in frequency (71.4%) and number (4.4). Mysidacea and Decapoda are secondary food items. Four specimens of *Erythrops* sp. were identified among Mysidacea. Decapoda, mainly *Processa* sp. and *Solenocera membranacea*, were observed only in summer.

From these data, the food of *B. apoda* appears to be a mixture of planktonic and benthic preys.

Reproduction

B. apoda is a viviparous species with sexual dimorphism and internal fertilization (Nielsen and Cohen, 1968). Morphological characteristics of the two sexes are well described in Papaconstantinou (1977) and Sardou (1980). Sex was determined in all but five specimens, and the sex-ratio (M/F) seems to be equal (1:0.98).

Ovaries are two paired elongate structures. In our dissected specimens, their length varies between 2.3 and 9.2 mm; the smallest ovaries contain oocytes in first stages of development, while the bigger have fewer oocytes, but in more advanced stages, or contain embryos. In the same ovary oocytes in different developmental stage can be found, also when embryos are present. Oocyte number ranges between 30 and 180 in both ovaries, depending on maturity stage. Four specimens had ovaries, with oocytes in developmental stage 2, quite different in shape, and the shorter and thicker gonad had more eggs than the other one. The oocytes are round shaped and orange-yellow in colour, in some cases with an oily spot. The spot diameter varies between 320 and 560 µm, according to oocyte size. It is quite different from the value (45 µm) found by Papaconstantinou (1977).

Spermatophores were found in 13 females with ovaries in different maturity stages. Spermatophores appear as long and narrow capsules (less than 100 µm measured along the major axis), containing spermatozoa (for detailed description see Nielsen *et al.*, 1968). In gonads quiescent or with eggs in an early stage of development, spermatophores form a compact mass stored in the caudal part of ovary. In a more advanced stage of ovary ripeness the mass of spermatophores is placed among the eggs. In spent females, the ovary looks like a sac with few undeveloped oocytes and residuals of spermatophores embedded in a gelatinous substance. This substance is probably an artefact due to a poor fixation of specimens (Nielsen *et al.*, 1968).

The reproductive cycle of *B. apoda* is not completely understood. The available sample covers all the size range of females, but specimens are scarce in autumn and winter (Table V). Even so, some conjectures can be made. As the smallest females in stage 1 (quiescent) were 30-32 mm SL and the smallest one in vitellogenic period (stage 2) was 34.8 mm SL, probably the former are still juveniles.

Females in gestation are present in January, June and August (Table V). The January specimen contains eggs with embryos in a very early developmental stage. In June embryos are in a more advanced stage, with the yolk sac still visible and a total length of

Taxa	$\bar{N}$	F %
Copepoda	4.4	71.4
Amphipoda	1.4	33.3
Mysidacea	1.4	23.8
Decapoda	1.2	23.8

Table IV. - Mean number ($\bar{N}$) and frequency of occurrence (F%) of identified food items in stomach contents of *Bellottia apoda*.

about 3 mm. In August, the two females in gestation period (stage 3) have all the embryos ready to hatch, with completely reabsorbed yolk sac and a TL of about 5 mm. These embryos near hatching show about 40 myotomes, and some spots of brown chromatophores are located dorsally and ventrally on each body side: 8-11 dorsally, 4-7 in

Table V. - Reproductive cycle of *Bellottia apoda*.

Month	N. of females	SL (mm) min - max	Ovarian maturity			
			Stage 1 (quiescency)	Stage 2 (vitellogenesis)	Stage 3 (gestation)	Stage 4 (recovery)
Jan.	4	35.0 - 40.0		3	1	
Feb.	-	---	---	---	---	---
Mar.	2	32.0 - 35.9	2			
Apr.	1	36.0				1
May	3	30.0 - 35.5	2	1		
Jun.	7	35.0 - 43.5		2	1	4
Jul.	2	34.8 - 48.0		1		1
Aug.	9	30.8 - 44.0	1	1	2	5
Sep.	-	---	---	---	---	---
Oct.	-	---	---	---	---	---
Nov.	1	38.2		1		
Dec.	-	---	---	---	---	---

Table VI. - Comparison between morphometric and meristic data of *Bellottia apoda* derived from previous literature and present work.

	Previous literature	Present work	
	min - max	min - max	min - max
		Males	Females
Standard length (mm)	25.5 - 60.0	25.9 - 42.8	32.0 - 48.5
Morphometric characters		***in % SL***	
Predorsal length	36.7 - 44.9	33.6 - 41.3	34.4 - 40.9
Preanal length	48.3 - 57.3	48.1 - 55.3	46.4 - 52.5
Head length	26.2 - 30.9	26.8 - 30.8	24.7 - 29.0
Jaw length	13.2 - 16.7	11.3 - 15.4	11.2 - 16.2
Orbit diameter	3.8 - 6.4	4.0 - 5.3	3.7 - 5.5
Interorbital width	5.4 - 9.5	6.0 - 7.4	5.8 - 7.8
Maximal body depth	13.4 - 22.1	18.7 - 24.4	19.4 - 23.1
Meristic characters			
Dorsal rays	81 - 97	81 - 95	81 - 96
Anal rays	61 - 80	65 - 76	64 - 76
Caudal rays	6 - 7	6 - 6	6 - 6
Pectoral rays	21 - 23	21 - 23	21 - 24

postanal position, 1 at level of anus and 3 before it. In dorsal and postanal regions, spots of the two sides seem to join together to form transversal bands. All but one female with spent ovaries were collected between June and August.

The above data indicate that summer is the main spawning season of *B. apoda.*

DISCUSSION

Our morphological data are in good agreement with those obtained from literature (Table VI). The sex-different relative growth in preanal and head length indicate sexual dimorphism, the head growing slower in females than in males. A difference in "preanal length" between the two sexes was previously reported in other brotulid fishes (Nielsen and Nybelin, 1963). Moreover, Hureau and Nielsen (1981) suggested the existence of sexual dimorphism in *Dicrolene pallidus* (Ophididae), based also on a different shape of the head.

The capture of 128 specimens of *B. apoda* leads us to believe that the species is not so rare as it seemed, at least in the Adriatic Sea. Its only nocturnal catchability and the great number of full stomachs suggest a nocturnal activity of the species.

Both the structures of dentition and gill rakers, as well as the stomach contents, show that *B. apoda* is a carnivorous predator. This confirms previous observations made by Nielsen and Cohen (1968) and Sardou (1980). The discovery of a food spectrum formed by planktonic and benthic organisms indicates a bentho-pelagic feeding behaviour of *B. apoda.*

Our data on reproductive cycle confirm the hypothesis that late summer is the main spawning season of *B. apoda* in the Adriatic Sea as in the Euboikos Gulf (Papaconstantinou, 1984). A compact mass of spermatophores was found in the posterior part of ovary in unripe females, and among the eggs as ripeness proceeds. This confirms the hypothesis of Nielsen and Cohen (1968) on capability of females to store spermatophores till complete ripening of eggs.

Apart from the specimen reported by Berdar *et al.* (1977), stranded on the beach of Messina, in the last 20 years all the specimens were collected on muddy or sandy-muddy grounds, at depths from 120 to 520 m. Type material was apparently collected in a quite different and shallower habitat (*Posidonia* bed, depth 30 m). The possibility of its mislabelling has to be considered. The hypothesis of Nielsen and Cohen (1968) that *B. apoda* lives in holes and crevices of rocks, based on Giglioli's original description, seems doubtful. At the same time, there is no evidence of a migration of mature specimens to the bathypelagic zone during the reproductive season as suggested by Papaconstantinou (1977). Probably *B. apoda* can hide, burying in the soft sediment or making burrows, but this hypothesis needs to be verified.

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